

## **Peanut/Tree Nut Desensitization and induction of tolerance in Children**

### **1 Proposed Research Questions**

1. What is the rate of desensitization in children allergic to peanuts or tree nuts?
2. Is the efficacy of a maintenance protocol of 300mg peanut protein similar to the efficacy of a maintenance protocol of 30mg peanut protein?
3. What are the predictors associated with success or failure of desensitization in children?
4. What are the molecular mechanisms involved in desensitization to peanut/tree nut?

### **2 Research Objectives and Significance**

**The investigators are proposing to initiate a study assessing a common desensitization protocol for peanut/tree nut allergy**

**This study would enable us to better determine the potential benefit of desensitization in individuals with peanut/tree nut allergy.**

More specifically, the investigators will address the following research objectives:

#### **Objectives**

- A. To develop a protocols for peanut/tree nut desensitization with high (300mg) and low (30mg maintenance dose).
- B. To determine the rate of desensitization to peanut/tree nut.
- C. To characterize predictors of successful desensitization.
- D. To characterize molecular mechanisms involved in the process of desensitization.

### **3. Gaps and Unmet Needs in Existing Knowledge on food desensitization**

### **3.i Why do the investigators need trials on food desensitization.**

Foods are reported to be primary inciting allergens for anaphylaxis and account for 33.2% to 56% of all anaphylaxis cases.<sup>(1-4)</sup> Further, food-induced anaphylaxis hospital admissions are reported to have increased, mainly in the first 2 decades of life.<sup>(5;6)</sup>

At present, the only treatment for food allergy is to avoid the allergy-causing food, while the only treatment for an allergic reaction is prompt administration of intramuscular epinephrine.<sup>(7;8)</sup>

Primary anaphylaxis prevention is based on allergen desensitization through immunotherapy and until recently was not available for food allergies. Although there are a few recent research protocols exploring desensitization for foods and although these have so far been largely successful,<sup>(9;10)</sup> up to this point there are no known guidelines describing the optimal candidate for desensitization nor are there criteria for the safest and most effective dosing schedule. Further, many of these studies have a very small sample size and do not use a control group. Hence, the efficacy of these protocols is hard to interpret<sup>(11-14)</sup> and additional larger scale randomized control studies are required.

### **3.ii Why peanut/tree nut .**

Peanut and tree nut allergies are the most common food allergies in children and adults in Canada affecting almost 2% and 1.5% of the population respectively.<sup>(15)</sup> and recent reports suggest that only 10%-20% will out-grow nut allergy.<sup>(16)</sup>

Further, peanut and tree nut allergy are most common trigger for anaphylaxis and the most common trigger for recurrent anaphylaxis.<sup>(17)</sup>

With the support of AllerGen NCE and CIHR the investigators have initiated in 2014 the first cross Canada study to assess desensitization to milk . This study now includes

almost 50 patients, and our preliminary results reveal an almost 80% success rate. In April 2016 in collaboration with Aimmune the investigators have initiated the first oral desensitization to peanut.<sup>(18)</sup> The investigators are now proposing to develop a protocol for peanut and tree nut desensitization based on the expertise, recruitment skills and data collected in our first 2 years of milk desensitization and more recently peanut desensitization.

Peanut and tree nuts are the most common foods causing acute allergic reactions and almost all fatal reactions in North America had been triggered by peanut or tree nuts. The term “tree nut” is collectively used to categorize nuts that grow on trees. Prevalence of individual tree nut allergies varies significantly by region, with hazelnut being the most common tree nut allergy in Europe, walnut and cashew the most common in the USA and Brazil nut, almond and walnut most commonly reported in Europe.<sup>(19)</sup> In Canada alone, recent research has shown up to 2% of children and 1% of adults have peanut allergy.<sup>(15;21)</sup>

The main peanut allergens are Arah1 and Arah2.<sup>(22)</sup> Most tree nut allergens identified to date are seed storage proteins such as the vicilins, legumins, arabinogalactans, and 2S albumins. Other tree nut allergens include profilins and hevein-related proteins, known for their contribution to the allergenicity of a wide variety of pollens, nuts, seeds, fruits and vegetables and their propensity for exhibiting a significant degree of IgE-mediated cross-reactivity.<sup>(23)</sup> Cross-reactivity among nuts may occur because of minor constituent panallergens such as profilins and lipid transfer proteins, or may involve major nut storage protein allergens, including albumins, legumins, and vicilins.<sup>(24)</sup> Strong cross-reactivity was present among walnuts, pecans, and hazelnuts, and mild cross-reactivity was present among cashews, Brazil nuts, pistachios, and almonds.<sup>(202)</sup>

Diagnosis of peanut/tree nut allergy is confirmed by having a convincing clinical history as well as the presence of a positive SPT defined as a wheal diameter at least 3 mm larger than that elicited by the negative control within 10 to 15 minutes of placement, provided by a physician, or an IgE level of at least 0.35 KU/L.<sup>(25)</sup> If however, exposure to

peanut/tree nut never occurred, or if clinical history is uncertain, diagnosis is based on having a positive SPT and a tree nut- specific IgE above 15 kU/L. <sup>(26)</sup>

Despite the importance of peanut and tree nut allergy, there are only three studies assessing oral immunotherapy for peanut and there is only one group that published to date a research desensitization to hazelnuts. No protocols have been published so far for other tree nuts. The protocol for hazelnuts reveals encouraging results with significantly increased thresholds to food induced allergic reactions after oral immunotherapy in the majority of those with hazelnut allergy<sup>(18;27;28)</sup> In conclusion, the efficacy of the immunotherapy, extent of desensitization versus tolerance, the quantity/ frequency of allergen consumption required to maintain this effect and molecular mechanisms involved in the desensitization process are currently unknown.

### **3. iii. Do the investigators achieve only desensitization or do the investigators induce also tolerance.**

Desensitization refers to a change in the amount of food antigen needed to cause allergic symptoms; this state is dependent on regular antigen exposure. In contrast, *tolerance* refers to long-term immunologic changes associated with the ability to ingest a food without symptoms and without ongoing therapy. Desensitization is a worthwhile therapeutic goal as it allows individuals freedom from the risk of accidental ingestion in everyday settings; achieving long-term clinical tolerance would allow safe food ingestion without ongoing therapy by inducing lasting immunologic changes.<sup>(28)</sup> Trials so far suggest that might be loss of tolerance within 48 h after elimination of the food from the diet.<sup>(30)</sup> However , these trials are flawed by their small sample size (3 patients).<sup>(30)</sup> In a recent study it was reported that prolonged ( at least 4 years) daily immunotherapy is

crucial for achieving Sustained Unresponsiveness (SU)=lack of dose limiting symptoms during DBPCFC and also 4-6 weeks after stopping OIT .<sup>(31)</sup>

### **3. iv Gaps related to the appropriate maintenance dose of peanut desensitization.**

The threshold doses of peanut and tree nut that trigger reactions ranges from 15 mg to 1000mg<sup>(32-34)</sup> and studies suggest that almost a third of products from Western Europe and two thirds of products from eastern Europe without precautionary labeling contain detectable levels of peanut or tree nut protein up to 245 mg per liter.<sup>(35)</sup> Hence it is crucial that desensitization protocols will be efficacious in protection against reactions to at least 300mg of peanut or tree nut protein .One previous study on peanut had shown similar efficacy in attaining sustained unresponsiveness (defined as no allergic reaction to 5 g of peanut ingested 1 month after stopping oral immunotherapy) between a protocol with 3000 mg maintenance dose versus 300mg.<sup>(36)</sup> It is of crucial importance to assess the efficacy of low dose protocols as trials aiming for high doses of peanut maintenance are associated with up to 40% drop-out due to adverse effects .

### **3.v Gaps regarding the roles of mast cells in desensitization.**

Given that food desensitization is a relatively rapid process, mast cell are implicated to have a key role in this process. Compared to activated cells, desensitized Mast cells were shown to have had impaired degranulation, calcium flux, secretion of arachidonic acid

products.<sup>(37)</sup> However, the effect of food desensitization on mast cells was not explored so far in clinical trials.

### **3vi. Gaps regarding the role of T regulatory cells in desensitization.**

Although several studies in humans report that food allergens induce specific effector T cells, there are significant controversies between studies regarding the T cell phenotype dominating that response. Some studies report a Th2 dominated response in children with food allergies whereas Th1- responses underlie oral tolerance <sup>(38)</sup>. Other studies suggest that food antigens induce a Th2 response regardless of the presence of food allergy<sup>(39)</sup>. Further, it is proposed that Treg cells may be crucial to control food related allergic reactions. A potential suppressive role of Treg in food allergies was exemplified in both animal models <sup>(40-42)</sup> and in human studies. Increased numbers of Treg cells in peripheral blood in children who outgrew allergy was reported <sup>(43;44)</sup> and decreased Treg suppression was associated with allergy in neonates <sup>(45)</sup>. Cord blood from offspring of atopic mothers showed fewer innate induced Treg cells and impaired suppression<sup>(46)</sup>. In line with these, an association with severe food allergy was described in patients with profound dysfunction of T reg cells that characterizes IPEX syndrome (immunodysregulation polyendocrinopathy enteropathy X-linked syndrome)<sup>(47)</sup>.

In contrast 1 study looking at the number of Tregs in 10 children undergoing desensitization reports no enhancement of CD4+CD25+Foxp3+ T(reg) levels in peripheral blood and suggests that there is unlikely a role for long-lasting systemic immunologic changes in desensitization. However, this study is limited by a small sample size and relatively short period of follow up ( 6 months). <sup>(48)</sup>

### **3.vii Gaps regarding the role of demethylation in desensitization.**

The rising incidence of food allergies is occurring more rapidly than changes to the genome sequence would allow<sup>(49)</sup> and recent studies suggest that epigenetic regulation (heritable changes in gene expression that occur in the absence of alterations in DNA sequences) may in part mediate the complex gene-by-environment interactions that can lead to asthma.<sup>(50)</sup> Experimental studies provide substantial in vitro data indicating that

DNA methylation of genes critical to T-helper cell differentiation may induce polarization toward or away from an allergic phenotype. Methylation of DNA and resulting changes in chromatin structure have been shown to initiate the process by which the Th cells lose their plasticity and differentiate productively toward the Th1 versus the proallergic Th2 pattern of cytokine gene expression.<sup>(51-53)</sup> So far no studies have explored the role of DNA methylation in short term processes such as desensitization for food allergens.

### **3viii Effect of Heat Treating Peanuts/Tree Nuts on Allergenicity**

There is ample evidence in the literature that there is a significant increase in the allergenicity of peanuts after undergoing thermal processing (i.e. roasting). Glycation at high temperatures via the Maillard reaction, the addition of amines on reducing sugars to provide Schiff bases and advanced glycation end products (AGEs), is proposed to be the primary mechanism of enhancement of allergic responses in roasted peanuts. Our primary objective is to develop strategies to decrease glycation and Maillard reaction products in the thermal processing of peanuts.

We propose to use High-Resolution Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy (HR-MAS NMR) to take an array of molecular snapshots of

intact raw peanuts from room temperature to roasted temperatures (i.e. 20-150°C) and to subsequently perform an antigen-specific ELISA, which measures IgE binding, to assess the allergenicity at each temperature. We will expand the approach to variable temperature studies under conditions where small molecules, such as sugars and amino acids, are removed from intact or macerated peanuts prior to heating.

#### **4. Methodological Approach/Study Design**

Seventy five males and females between 2 to 40 years of age, diagnosed with peanut /tree nut allergy will be approached by our study group. Participants will be recruited from the allergy clinic at the Montreal Children's Hospital and at collaborating sites in Toronto and Vancouver **Participants who meet inclusion criteria will be enrolled in a desensitization programme specific to their allergy. Thus a peanut allergic participant will be desensitized to peanut only, while a patient allergic to cashew for example, will be desensitized to cashew only. Patients with more than one peanut or nut allergy will be offered the choice of to which allergen they would prefer to be desensitized**

**Inclusion criteria:** Children and adults between 2 and 40 who satisfy all the following criteria will be included:

- A history suggestive of immediate allergy to peanut/tree nut . A convincing clinical history of an IgE mediated reaction to a specific food will be defined as a minimum of 2 mild signs/symptoms or 1 moderate or 1 severe sign/symptom that was likely IgE mediated and occurred within 120 minutes after ingestion or contact (or inhalation in the case of fish and shellfish).

Reactions will be considered mild if they involve pruritus, urticaria, flushing,



or rhinoconjunctivitis; moderate if they involve angioedema, throat tightness, gastrointestinal complaints, or breathing difficulties (other than wheeze); and severe if they involve wheeze, cyanosis, or circulatory collapse.<sup>(54-57)</sup>

- The presence of at least one of the following confirmatory tests:
  - Positive SPT to peanut/tree nut or its proteins (weal diameter 3 mm larger than that of the normal saline control). The allergens used will be commercial extracts of peanut/tree nut (Omega Labs, Toronto, Ontario).
  - Detection of serum specific IgE (>0.35 kU/L) to peanut/tree nut or any of its proteins, measured by fluorescence enzyme immunoassay (Phadia, CAP System, Uppsala, Sweden).
  - Positive oral challenge test to peanut/tree nut. Oral challenges will be performed with raw peanut/tree nut according to the recommendations of the position paper of the European Academy of Allergy and Clinical Immunology<sup>(58)</sup>.
- Informed consent form signed by the parents or legal guardian (appendix B).

**Exclusion criteria.**

- Patients who are unstable from a respiratory point of view ..
- Patients who present with intercurrent disease at the time of starting desensitization.
- Non-IgE-mediated or non-immunological adverse reactions to nuts.

- Malignant or immunopathological diseases and/or severe primary or secondary immune deficiencies.
- Patients receiving immunosuppressor therapy
- Patients receiving  $\beta$ -blockers (including topical formulations).
- Associated diseases contraindicating the use of epinephrine: cardiovascular disease or severe hypertension.
- Patients diagnosed with eosinophilic gastrointestinal disorder .

### **STUDY PROCEDURES**

The desensitization protocol will be performed at the Center of Innovative medicine (CIM ) as is currently done for our peanut desensitization study, under the direct supervision of the medical and nursing staff, and with full cardiopulmonary resuscitation measures available for the treatment of possible allergic reactions that could occur during the procedure. Oral and intravenous doses of diphenhydramine and intramuscular epinephrine were at the bedside at all times.

#### **Desensitization to peanut (B1 and B2).**

#### **Oral Food Challenge**

Patients will begin with a blinded, placebo-controlled oral food challenge (BPCFC) to peanut that will take place over two days, with patients receiving doses of placebo one

day and peanut/tree nut protein the other. In all cases, protein will be in the form of crushed peanut/tree nut. All challenges and desensitization procedures will be done with commercially available peanut/tree nut proteins. Patients and evaluating physicians will be blinded, but not the study nurses nor the study coordinator. On both days, patients will arrive at the Center for Innovative Medicine (CIM) in the morning. The CIM services include equipment and medications required to monitor and treat potential reactions. The CIM nurse that will assist in the desensitization protocol is a nurse that has been trained to work in an intensive care unit. Patients will be reminded beforehand that they must be in good health for the challenge. If they are not in good health, the challenge will be rescheduled. Upon arriving, a physical exam will be performed and vital signs taken. Skin prick test to peanut/tree nut will be performed to establish baseline values in both. An IV catheter will be inserted in the patient's arm. The doses will be mixed in a food vehicle tolerated by the participant, such as pudding or fruit puree, that the participant is known to tolerate. Doses will be given by the study nurse every 15-20 minutes, depending on patients' tolerance of the dose (see Appendix B for challenge doses). If participants tolerate the last, 300 mg dose, they will be given a dose of food that contains the allergen in question – peanut butter in the case of a peanut challenge, Nutella in the case of hazelnut allergy. The study nurse will be with the participant for the duration of the challenge and as soon as the participant demonstrates objective signs of an allergic reaction, the challenge will be stopped and the reaction treated. Participants will be kept under observation in the CIM for two hours after the resolution of the last symptoms.

Given the similarity of allergens between walnut and pecan and cashew and pistachio<sup>(202)</sup>, the investigators will offer challenge with the two cross reacting allergens ( 1 week to 4 weeks apart ) for participants who report an allergy to at least one of these allergens .

### **Randomization**

Through a process of stratified randomization (according to sex), participants will be randomly assigned in a 1:1:1 fashion to one of 3 study groups. The first group : group A will receive active desensitization with a maintenance dose of 300 mg , the second, group B will receive active desensitization with a maintenance dose of 30 mg , and the third group C group will not receive any treatment but will be followed up regularly ( appendix B protocols ) . Patients in groups A and B will be blinded to their group assignment. Participants randomized to group C will be offered the opportunity to enter the desensitization programme after their year of observation ends. They will be randomized in a 1:1 fashion to either the 300 mg protocol or the 30 mg protocol.

### **Rush Desensitization**

The investigators will base our proposed desensitization protocol on previous studies by our group on a peanut desensitization protocol that has been active in the last year at the Montreal Children's Hospital. The modified rush two days will begin with a first single dose of 0.5 mg of peanut/tree nut protein. After the initial dose, subjects will receive approximately doubling doses every 30 minutes until a dose of 5 mg. All doses will be mixed with an acceptable vehicle food chosen by the subject and his or her parent. Those who tolerate 5 mg , will be asked to return the next day for one dose of 3mg. If

this is tolerated, participants will be supplied with enough peanut/tree nut protein to be able to take a daily dose of 3 mg for the following 2 weeks and will come to the Montreal Children's hospital after at the end of these two weeks for a dose of 6 mg (doses will be aliquoted and put in capsules by a professional pharmacist).. Self-administered epinephrine will be provided to all patients' caregivers, along with instructions and indications for administration and education about the nature of possible reactions to OIT. **Patients who are unable to complete the rush desensitization will not be eligible to continue the study**

Successful desensitization will be defined when the patient will be able to tolerate 300mg of peanut/tree nut protein. A slow regimen will be used in those patients who will experience repeated moderate reactions or any severe reaction to a given dose; that regimen will consist of reducing the dose to the previously tolerated dose for 1-2 weeks or splitting the dose into two equal portions according to the discretion of the PI or co-PI.<sup>(11)</sup>

SPT with the allergens listed above will be performed on all patients before starting desensitization, and the tests will be repeated at 3, 6 and 12 months. Blood tests will be also performed to measure sIgE and sIgG, T cell phenotypes (including regulatory Tcells), vitamin D levels and DNA methylation before desensitization, at 3 weeks and at 3, 6 and 12 months.

Reactions during the desensitization protocol will be classified according to the categories proposed by Perry et al.<sup>(59)</sup>: mild reactions are defined when symptoms are limited to the oral mucosa or the skin; severe reactions include cardiovascular or

respiratory symptoms or involvement of any four systems; all other reactions will be classified as moderate, although the investigators will consider isolated abdominal discomfort as mild when it lasted for 30 min or less.

The protocol will be individualized according to each patient's tolerance to peanut/tree nut:

- Mild allergic reactions will be treated when necessary and the regimen will be continued when the patient becomes asymptomatic. The previously tolerated dose will be repeated before resuming the process.
- Moderate reactions will be treated and desensitization would be restarted on the following day at the previously tolerated dose.
- Severe reactions will be treated with the necessary measures and in the appropriate department, followed by an assessment of whether to interrupt desensitization or reduce the dose on the following day to 1/10 of the dose that caused the reaction.

### **Build-up phase**

Two weeks after the two-day rush Desensitization, participants will begin the build-up phase. Participants will come to the CIM every two weeks to receive their first dose at each level. Participants will then consume this dose at home for two weeks, then return to the CIM for the next dose augmentation (doses are listed in Appendix B). Home doses will be provided in individual preweighed containers, and subjects will be instructed to ingest 1 dose daily for 2 weeks. Dose escalations will be made at visits in the research unit to ensure that subjects tolerate increases, and subjects will be observed for at least 2 hours after increases for signs of clinical reactions. This process will be repeated for 11

doses (22 weeks) with almost doubling of the dose in each hospital visit till a dose of 300mg is reached for group A. For group B participants will attain 30 mg after 6 weeks and will continue with the same dose for 16 weeks

#### **Follow-up / Maintenance phase**

In order to compare the efficacy of a 30 mg peanut protein dose to that of a 300 mg dose in inducing peanut/tree nut desensitization, participants in Groups A and B will both undergo DBPCFC at similar time points during the maintenance phase. The first of these challenges will take place one year after beginning desensitization. Participants in either group whose progress is delayed because of symptoms during the escalation phase will undergo their DBPCFC three months after attaining their maximal dose (either 30mg or 300mg). The subjects will be also instructed to otherwise continue a peanut/ tree nut-elimination diet for the duration of the study.

Subjects and families will be asked to complete daily home diaries to document that daily doses were taken, as well as to report accidental ingestions, problems with dose administration, or related symptoms. Follow-up visits in addition to or in conjunction with the biweekly visits for dose escalation will be planned at 3, 6, 9, 12 months from enrollment. Each visit will involve a medical history and physical examination. SPT, serum tree nut-specific IgE and IgG concentrations, T cell phenotyping and DNA methylation will be measured at enrollment and at follow-up visits. At the 9-month follow-up visit, subjects from both groups will undergo another DBPCFC, similar to that done following escalation.

The patients' parents will be instructed verbally and in writing about the recommendations to be followed after desensitization and how to treat possible allergic

reactions. They will also given a direct telephone line to members of the study staff for consultation. Patients will be told not to perform physical exercise <sup>(60;61)</sup> for 2 h after eating and not to take non-steroidal anti-inflammatory drugs for 3 h before or after ingestion. No special recommendations will be given for viral infections.<sup>(11)</sup>

### **Food challenges**

As indicated above, subjects who tolerate 300mg (group A) or 30mg (group B) of peanut/tree nut will have a two more BPCFC to determine their allergenic reactivity to peanut protein. The first of these challenges will take place one year after they begin desensitization. Participants in either group whose progress is delayed because of symptoms during the escalation phase will undergo their DBPCFC three months after attaining their maximal dose (either 30mg or 300mg). . Subjects will continue OIT dosing through the day before the challenge. One challenge will consist of doses of peanut/tree nut protein given every 10 to 20 minutes starting at 1 mg in increasing amounts up to a total of 2044mg of peanut/tree nut protein (See Appendix B, section A1 for doses). The other challenge will consist of placebo given also in 6 doses. All negative challenge results will be confirmed by means of open challenge, with at least three peanuts/tree nuts provided 1 hour after completion of the double-blind portion. The second DBPCFC will take place nine months into the Follow-Up/ Maintenance phase. Participants who pass these challenges will be instructed to continue to ingest the maintenance dose daily to the end of the follow-up period.

Participants in Group B who have allergic reactions during this oral food challenge will re-entered into the Escalation Phase and proceed to the 300 mg dose.



At the end of the study, oral desensitization will be offered to the control patients who had not achieved tolerance.

Participants who had challenges to both cashew and pistachio will repeat the challenges to both nuts at this time point. This will be done to assess whether desensitization to one of these nuts confers protection to the other, antigenically-similar nut

**Variables measured.** The following variables will be measured in each patient:

A. The primary study variable will be the presence of desensitization defined by the ability to tolerate peanut/tree nut 1 year after the start of the trial. This is a qualitative variable with three categories: total desensitization (300mg of peanut/ nut protein); partial desensitization (30-300mg of peanut/ nut protein); and failed desensitization (<30 mg of peanut/ nut protein). Only patients presenting total desensitization will be regarded as being successfully desensitized.

B. Secondary variables will be:

1. Number and severity of adverse reactions occurring after oral exposure to peanut/tree nut during the desensitization and follow-up phases.

2. Minimum dose of peanut/tree nut that triggered symptoms during the desensitization protocols or challenge tests.

3. Days until desensitization was achieved, excluding weekends.

4. Indices of desensitization :

SPT weal size and levels of sIgE and sIgG before desensitization, during follow-up and at the end of the study.

5. Mast cell activation.

Mast cell activation will be assessed through a proxy measure :the basophil activation test. This test is based on the percentage of CD63-expressing basophils detected by flowcytometry . This test is done by incubating basophils from highly sensitized atopic donors or preferably after priming them with Il-3 ( regardless of basophil source ) <sup>(62;63)</sup> with sera from patients . The investigators will assess the expression of CD63 on basophiles after addition of tree nut/ antigens respectively as well as an irrelevant antigen ( gliadin and tetnus toxoid) The expression of CD63 on these cells is compared to values acquired after incubation with healthy controls and the same antigens. Values above the mean of CD63+ cells + 2 SD induced will be considered as positive. <sup>(64)</sup>

6. Indices of T cell tolerance :

Levels of Treg and cytokine release at base line and during desensitization. In order to characterise molecular pathways associated with desensitization , the investigators will draw 5 cc of blood from each participant and check their T cell phenotype including their Treg cells and the response to peanut/tree nut protein. As control the response to an irrelevant antigen (gliadin) and to tetanus toxoid will be assessed. The investigators will invite participants for annual follow ups in the first 5 years after study entry. The investigators will also offer the desensitization to the control group at the end of the study.

**7. Health-Related Quality of Life (HRQL) can be measured in food allergy and was validated for both children and adults.<sup>(65;66)</sup> Given that HRQL measurements may be used to measure the effects of an intervention on the patient's quality of life,**

**patients will be asked to fill a validated HRQL for children ( up to 18 years )<sup>(65)</sup> at study entry and after completing 12 months of maintenance**

## **5. Statistical Considerations**

Given that previous studies in adults have attributed at least 50% improvement in the ability to tolerate peanut/tree nut,<sup>(67)</sup> with an  $\alpha$  of 0.05 and a power ( $1 - \beta$ ) of 0.80, a sample of 17 cases for group A , 17 for group B and 17 controls will be recruited. Statistical analyses will be performed using paired t-tests. A P value of  $<0.05$  will be considered significant.

Descriptive statistics of the variables including means, standard deviations and frequencies will be computed for all study variables. The concentrations of sIgE and sIgG , the weal size , Mast cell, T cell and DNA methylation status **and HQRL scores** at the different study time-points will be compared with their baseline values using the one-sample paired t-test and to the control using a 2 sample t-test .

Multiple regression models will be used to assess factors associated with response to desensitization (i.e. baseline demographic characteristics; gender; age; baseline basophile activation; T cell status, baseline SPT and specific IgE, DNA methylation status, vitamin D status and season).

## **6. Ethical Considerations**

In the consent form given at study enrolment, participants will be advised that they will be randomly assigned to either desensitization treatment or observation alone for one year, (appendix B). Participants will also be advised that their data will be shared only among the study members. Participants who will be at the control group will be told that they will be offered the desensitization protocol one year after study entry.

## **7. Confidentiality and Data Management**

All research documents (case report forms, questionnaires, etc) will be stored in a locked cabinet in the Centre for Innovative Medicine (CIM) at the Glen. This facility has limited access, such that non-research personnel cannot access the area.

Electronic study records will be kept on a password-protected computer in the CIM. All participants will be assigned a unique study number and study information will be entered under this study number. The key linking the participant and their study number will be kept by the principal investigator.

Once the study has been completed, all physical study documents will be archived for 25 years at Iron Mountain. Electronic information will be stored by the Principal Investigator for 25 years

## **8. Research Deliverables and Milestones**

<b>Timeframe</b>	<b>Deliverables</b>	<b>Milestones</b>
08/16 – 03/ 17	Develop sampling frame; ethics approval;	Ready to recruit participants
05/17 –08/19	Collect data from participants	12 weeks of data collection in

treated participants completed

08/19– 12/23

Knowledge translation activities:

Preparation of abstracts and

dissemination workshops &amp; manuscript

manuscript

preparation

### 9. Requirement for networking across Disciplines and Sites

Our researchers have a legacy of working across disciplines, across sites, and across sectors. For this study, the investigators have assembled a multi-institutional research team with cross-disciplinary expertise Dr Ben-Shoshan (Montreal Children's Hospital, McGill University) and Dr Mazer and Dr McCusker (Montreal Children's Hospital ) bring expertise in clinical immunology to the research team. Dr. Ann Clarke (Montreal General Hospital) will bring expertise in the field of clinical allergy and together with Dr. Ben-shoshan, will assisted with the study design and will supervise the statistical analyses. Epidemiologic and biostatistician skills are essential to undertaking this program of research and are represented in the persons of Moshe Ben-Shoshan (McGill) Msc in Epidemiology and Mr . Duncan Lejtenyi. Mr Duncan Lejtenyi will also be responsible for the laboratory evaluation of CD63 levels. Finally, as incorporated in our protocol, the investigators will share the results of this research with representatives of key constituencies involved in the data collection –patients with food allergies and

their families, physicians and allied professionals (e.g. nurses) through scientific meetings, our the investigators site and publications in medical journals.

### **10. Organization of Research Team**

The nominated principal applicant, Dr. Moshe Ben-Shoshan, and co-principal applicants, Dr. Bruce Mazer, will oversee all aspects of the proposed research. The collaborators, Dr Stark , Dr Piccirillo and Dr Bar-Or will aid in the recruitment of patients and the assessment T cells and DNA methylation status respectively. Dr Julia Upton from Sick Kid's Hospital in Toronto and Dr Edmond Chan at BC Children's Hospital are external collaborators and will recruit patients from their respective centers

Mr Duncan Lejtenyi , who had worked with our team in numerous studies will serve as research coordinator.

### **11. External Research Partnerships and funding**

As indicated above, external research partnerships are considered essential to the activities of our research team. The investigators will apply to receive substantial financial support from the CIHR and from AllerGen (appendix D budget). The investigators will work closely with client organizations such as the Anaphylaxis Canada , AAIA ( allergy/Asthma Information Association ) , and the AQAA (Association québécoise des allergies alimentaires ). These support a cyber-society of people with food allergies and will provide support for our research activities from their limited budgets, again indicating the relevance of our research efforts to their needs.

<b>Name of Partner</b>	<b>Nature of participation</b>	<b>Contribution (CA\$)</b>	<b>Letter</b>
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				yes /no
		Cash	In-kind	
Anaphylaxis			1000	Y
Canada				
AAIA			1000	Y
AQAA			1000	Y
<b>Total value of partnerships</b>				

## References

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**Appendix A : Disease burden and gaps.****Table 2. Primary and secondary prevention measures for food allergies<sup>a</sup>**

Trigger	Primary prevention	Route of desensitization	Reference number	Secondary prevention
<b>Foods</b>		PO, SL	(14;68-70)	Avoidance of
	Tree nut	PO	(11-13)	education of allergic
	Peanut	PO	(27)	their care-givers
	Tree nut	SL	(29)	avoidance, improvement
	Peach	SL	(71)	prepackaged food wearing of Medication specific food allergy

<sup>a</sup> only the most common foods , drugs and insect desensitization approaches are mentioned.

PO, **Per os**; SL, **Sublingual**; SC,

**Table 2.Environmental factors associated with food allergy.**

Factor	study	Type of study	Effect
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Season	Vassall o MF	Case control	Children younger than 5 years born in fall or winter had higher odds of food allergy compared with controls.
Drugs	Palli- Scholl	Case control	The relative risk to develop food-specific IgE after anti-therapy was 10.5 (95% CI,1.44,76.48).
Microbial exposure	Gourbe yre	Review of case control and cohort studies	No clear conclusion regarding probiotic beneficial effect on the prevention or treatment of allergy .
Food consumption (quantity and timing)	Poole JA	Cohort	After adjusting for breastfeeding duration, introduction of cereal, family history of allergy, and history of food allergy at 6 months of age, age at initial exposure to cereal grains correlated to be strongly associated with wheat allergy ( $\geq 7$ months: adjusted OR: 3.8; 95% CI, 1.18,12.28)
	Du Toit	Case control	After adjustment for atopy, other food allergies, age, and sex, the RR for peanut allergy in the UK vs Israel is 5.8 (95% CI, 2.8,11.8), and largest and most significant difference in allergy between the UK and Israel was observed in the age of introduction of peanut ( $P < .0001$ ). By 9 months of age, 69% of Israelis were eating peanut compared with only 10% of UK infants.
	Katz	Cohort	The OR was 19.3 (95% CI, 6.0,62.1) for development of cow-mediated CMA among infants with exposure to cow protein at age of 15 days or more ( $P < .001$ ) vs those introduced to cow protein before 15 days.



	Joseph	Cohort	Early feeding reduced the risk of peanut sensitization among children with a parental history [adjusted OR, 0.2 (95% CI, 0.1,0.7); P = .007]. The relationship also became significant for when a cutoff for IgE of $\geq 0.70$ IU/mL was used [adjusted OR, 0.5 (95% CI, 0.3,0.9)].
	Koplin	Case control	Introduction of cooked at age 4 to 6 months, vs later exposure reduced the risk of allergy [OR, 0.2 (95% CI, 0.06-0.71)].
	Des Roches	Case control	The reported consumption of peanuts during pregnancy and breastfeeding was higher in the case group (those who developed peanut allergy and associated with an increased risk of peanut allergy in offspring [OR, 4.22 (95%CI, 1.57,11.30) and OR, 2.11 (95% CI, 1.31,3.97) for pregnancy and breastfeeding, respectively].
	Sichere r	Case control	Multivariate analysis including clinical, laboratory, and demographic variables showed frequent peanut consumption during pregnancy {OR, 2.9( 95% CI, 1.7,4.9)] to be associated with peanut IgE $\geq 5$ kUA/L.
Food processing	Chung	Laboratory analysis	After curing and roasting, mature peanuts exhibited approximately 20% higher levels of advanced glycation end products adducts and higher IgE binding vs immature peanuts.
	Yadzir	Laboratory analysis	Extracts from raw shrimp bound higher IgE than extracts from boiled shrimp, but the purified boiled tropomyosin (the major shrimp allergen) demonstrates higher IgE binding vs raw shrimp.

	Samson	Laboratory analysis	Thermal processing can lead to the formation of new and complex structures.
Vitamin D	Milner	Cohort	Early vitamin D use (within the first 6 months of life) was associated with a higher risk for food allergies in the exclusively formula-fed population [OR,1.63( 95% CI,1.21,2.20)]. Vitamin D use at 3 years of age was associated with increased risk for food allergies but not asthma in both breastfed [OR,1.62(95% CI,1.19,2.21) ]and exclusively formula-fed infants [OR, 1.57( 95% CI,1.03,1.88)].
	Cramarow	Ecologic study	Strong north-south gradient for the prescription of EpiPen in the United States, with the highest rates found in New England [adjusted $\beta$ for New England vs the rest of the US, 4.07 (95% CI, 2.77,5.36)]
	Mulins et al	Ecologic study	Using multivariate analysis , EpiPen prescription rates were higher in southern latitudes (less sunlight) compared with northern regions [ $\beta$ , -19.22( 95% CI, -26.71 , -11.73)].
	Mulins et al	Ecologic study	Southern latitudes were associated with higher hypoallergenic formulae prescription rates [beta, -147.98( 95% CI,-281.83,14.14)].

OR, odds ratio;RR, Relative Risk; CI,confidence interval ;CMA, Cow's Allergy

**Appendix B. protocols.****A1. BPCFC**

*Double-blind, placebo-controlled food challenge (BPCFC) up to 100 mg (144 mg cumulative) done at screening and 3 months post OIT*

<b>Dose</b>	<b>Cumulative</b>
1 mg	1 mg
3 mg	4 mg
10 mg	14 mg
30 mg	44 mg
100 mg	144 mg
300	444

**A 2. Scale for Grading Reaction Severity**

<b>Score</b>	<b>Symptom</b>	<b>Action</b>
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<b>Mild</b>	Pruritus, Urticaria, Flushing, Rhinoconjunctivitis	Observe May give Antihistamine (e.g. Benadryl or Reactin as prescribed) <b>Call Research Team</b> Research team will evaluate if dose adjustment is needed and if next dose will be given at home or in hospital.
<b>Moderate</b>	Angioedema, Throat tightness, Gastrointestinal complaints (cramping, . pain,vomiting,diarrhea) Respiratory symptoms (Cough, Mucous production)	Give epinephrine IM as per protocol Give Antihistamine (e.g. Benadryl or Reactin as prescribed) Seek urgent care (hospital emergency room) Call Research team To give next adjusted dose in hospital research unit (CIM)
<b>Severe</b>	Wheeze, Respiratory Distress Hypoxia, Cyanosis, Hypotension Circulatory collapse (Shock)	Give epinephrine IM as per protocol Give Antihistamine (e.g. Benadryl or Reactin as prescribed) Call 911 Seek urgent care (transfer to hospital emergency room) Call Research team; if the symptoms are not improving within 10 minutes of the first dose, instructions will be given from the team regarding use of a second dose of epinephrine.

**B1. Initial Escalation: Day 1 and 2**

- **Verify no wheezing, flare of atopic disease, or other intercurrent illness**
- **Update history and con meds**
- **Vital signs and PE**
- **PEFR**
- **Dose escalation at 20 minute intervals**
- **Post-dose vital signs**

- Vital signs q30min x3 post last dose
- Monitoring for AEs (see

Day 1	Day 1	Day 2	Day 2
Dose ( raw peanut/ tree nut)	Dose ( crushed peanut/ tree nut)	Dose( raw peanut/ tree nut protein)	Dose( crushed raw peanut/ tree nut)
0.5mg	2.0 mg	3mg	
1mg	4.0 mg		
1.5mg	6.0 mg		
2.5 mg	10.0 mg		
3mg	12 mg		
3.5mg	14 mg		
4mg	16 mg		
4.5mg	18 mg		
5mg	20 mg		

#### B2a. Build up phase for 300mg (group A)

Dose Number	Dose (peanut/tree nut protein) (mg)	Dose (crushed peanut/tree nut (mg)	Interval (weeks)	Percent Increase from Previous Dose
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<b>1</b>	<b>3</b>		<b>2</b>	
<b>2</b>	<b>6</b>	<b>24</b>	<b>2</b>	<b>100%</b>
<b>3</b>	<b>12</b>	<b>48</b>	<b>2</b>	<b>100%</b>
<b>4</b>	<b>20</b>	<b>80</b>	<b>2</b>	<b>67%</b>
<b>5</b>	<b>40</b>	<b>160</b>	<b>2</b>	<b>100%</b>
<b>6</b>	<b>80</b>	<b>320</b>	<b>2</b>	<b>100%</b>
<b>7</b>	<b>120</b>	<b>480</b>	<b>2</b>	<b>50%</b>
<b>8</b>	<b>160</b>	<b>640</b>	<b>2</b>	<b>33%</b>
<b>9</b>	<b>200</b>	<b>800</b>	<b>2</b>	<b>25%</b>
<b>10</b>	<b>240</b>	<b>960</b>	<b>2</b>	<b>20%</b>
		<b>1200</b>	<b>Enter</b>	<b>25%</b>
<b>11</b>	<b>300</b>		<b>Maintenance Period</b>	

**B2a. Build up phase for 30mg (group B)**

	<b>Dose</b>	<b>Dose</b>		
	<b>peanut/tree</b>	<b>crushed</b>		<b>Percent</b>
	<b>nut protein</b>	<b>peanut/tree nut</b>	<b>Interval</b>	<b>Increase from</b>
<b>Dose Number</b>	<b>(mg)</b>	<b>(mg)</b>	<b>(weeks)</b>	<b>Previous Dose</b>
<b>1</b>	<b>3</b>	<b>12</b>	<b>2</b>	

<b>2</b>	<b>6</b>	<b>24</b>	<b>2</b>	<b>100%</b>
<b>3</b>	<b>12</b>	<b>48</b>	<b>2</b>	<b>100%</b>
<b>4</b>	<b>20</b>	<b>80</b>	<b>2</b>	<b>67%</b>
<b>5</b>	<b>30</b>	<b>120</b>	<b>2</b>	<b>50%</b>
<b>6</b>	<b>30</b>	<b>120</b>	<b>2</b>	<b>00%</b>
<b>7</b>	<b>30</b>	<b>120</b>	<b>2</b>	<b>0%</b>
<b>8</b>	<b>30</b>	<b>120</b>	<b>2</b>	<b>0%</b>
<b>9</b>	<b>30</b>	<b>120</b>	<b>2</b>	<b>0%</b>
<b>10</b>	<b>30</b>	<b>120</b>	<b>2</b>	<b>0%</b>
		<b>120</b>	<b>Enter</b>	<b>0%</b>
<b>11</b>	<b>30</b>		<b>Maintenance Period</b>	

If the subject does not tolerate a given dose and symptoms are mild, then that dose or the previously tolerated one is repeated, and the protocol proceeds as outlined. If the subject experiences significant symptoms, then the protocol is stopped, and the highest tolerated dose is used as the starting daily dose.

All doses will be pre-weighed and encapsulated to blind both patients and evaluating physicians

After achieving a dose of 300 mg(group A) or 30 mg (group B) , the subject will return for a first maintenance visit 2 weeks later.

- Visits are then every 4 weeks until Exit Visit

B3.Challenges during maintenance period ( for group A or B):

Dose (peanut protein)	Dose (crushed peanut)	Cumulative (peanut protein)	Cumulative (crushed peanut)
3 mg	12 mg	3 mg	12 mg
10 mg	40 mg	13 mg	52 mg
30 mg	120 mg	43 mg	172 mg
100 mg	400 mg	143 mg	572 mg
300 mg	1200 mg	443 mg	1772 mg
600 mg	2400 mg	1043 mg	4172 mg
1000 mg	4000 mg	2043 mg	8172 mg

#### Appendix C. Budget

Total budget estimate: 78,000CAD per year including:



	Estimate (CAD)	comment
Yearly salary for an undergraduate student for salary that will support training in Lab evaluation mechanisms <b>for tolerance detection</b>	35,000	
Study coordinator	40,000	Yearly salary with benefits
Paediatric test center (70CAD for each blood sample)	3000	
Staining with monoclonal Abs	3000	
Use of FACS	3000	
CBA	3500	
Culture materials and stimulating material	3500	
Travel expenses for presentation of results in scientific conferences	5000	
	78,000	yearly

Appendix D: Flow chart of study procedures





